



### Supplementary Figure 1: HDACi Panabinostat and Valproate have no effects on AtT-20

**cell viability; normal corticotrophs are resistant to SAHA.** No difference was found between treatment and control groups in the viability of AtT-20 cells, as examined by MTT assay, after 24 h exposure to SAHA, Valproate, and Panobinostat (**A**). Normalized serum ACTH levels in nude mice treated with various concentrations of SAHA (0.5 to 2 mg) are not significantly affected after 3 h (**B**). Retinoic acid (10nM) reduced by 6% ACTH secretion by AtT20-cells compared to 58% when exposed to SAHA (2.5 µM) (**C**). The AtT-20/D16/16 cell strain demonstrates increased COUP-TF1 expression levels (**D**). Horizontal bars represent mean ± standard deviation. ACTH – adrenocorticotrophic hormone, COUP-TF1 – chicken ovalbumin upstream promoter transcription factor 1.

Gene	Primer sequence
$\beta$ -actin	Forward: 5'-TTCTTTGCAGCTCCTTCGTTGCCG; 3' Reverse: 3'-TTTGCACATGCCGGAGCCGTT-5'
bax	Forward: 5'-CAAACCTGGTGCTCAAGGCC-3' Reverse: 3'-GCACTCCCGCCACAAAGAT-5'
bcl-2	Forward: 5'-CCGGGAGAACAGGGTATGAT-3' Reverse: 3'-CACCTTCTCCCAGCCCTTCG-5'
LXR $\alpha$	Forward: 5'-GCGTCCATTCAGAGCAAGTGT-3' Reverse: 3'-TCACTCGTGGACATCCCAGAT-5'
RXR $\alpha$	Forward: 5'-GACATACGTGGAGGCAAACATG-3' Reverse: 3'-GCTGCTTGACAGATGTTGGTAAC-5'
POMC	Forward: 5'-GTTCAAGAGGGAGCTGGAAGGCGA-3' Reverse: 3'-TTGCTCCAGCGGAAGTGCTCCA-5'

**Supplementary Table 1. Primer sequences used for RT-qPCR.**

## Supplementary Materials and Methods

### *Nucleic acid extraction and amplification*

DNA was extracted and purified with DNeasy Blood and Tissue Kit (Qiagen, Germany) following the manufacturer's protocol from the adenomas obtained intra-operatively. DNA was subsequently amplified using intron-spanning USP8 primers(1). PCR was carried out with

Platinum PCR SuperMix (Thermo Fischer Scientific, USA) according to the manufacturer's recommendations. Briefly, reaction was performed on 200 ng of DNA in a final volume of 50  $\mu$ l containing 22 U/mL recombinant *Taq* DNA polymerase complexed with *Taq* antibody, 22 mM Tris-HCl (pH 8.4), 55 mM KCl, 1.65 mM MgCl<sub>2</sub>, 220  $\mu$ M dGTP, 220  $\mu$ M dATP, 220  $\mu$ M dTTP, and 220  $\mu$ M dCTP. Thermocycling conditions were with an annealing temperature of 58 °C. Lastly, agarose gel (2%) electrophoresis confirmed the presence of a single band following PCR.

#### *USP8 sequencing*

Direct PCR products were sent for sequencing using Eurofins Genomics sequencing services. Primer sets for USP8(1) were submitted with samples and further utilized for sequencing.

#### *USP8 sequence alignment*

Sequence similarity analysis was performed using the Basic Local Alignment Search Tool (BLAST)(2) of the National Center for Biotechnology Information (NCBI). Specifically, all human USP8 transcript variants (XM\_006720761.3, XM\_011522193.2, XM\_006720762.3, XM\_017022718.1, XM\_017022719.1, XM\_017022720.1, XM\_017022721.1, XM\_017022722.1) were aligned to sequenced patients' DNA samples.

### **References**

1. **Reincke M, Sbiera S, Hayakawa A, Theodoropoulou M, Osswald A, Beuschlein F, Meitinger T, Mizuno-Yamasaki E, Kawaguchi K, Saeki Y, Tanaka K, Wieland T, Graf E, Saeger W, Ronchi CLC, Allolio B, Buchfelder M, Strom TM, Fassnacht M, Komada M, Buchfelder M, Fassnacht M, Masayuki Komada.** Mutations in the deubiquitinase gene USP8 cause Cushing's disease. *Nat. Genet.*

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2. **Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ.** Basic local alignment search tool. *J. Mol. Biol.* 1990;215(3):403–10.